Renal Tumors and Other Lesions in Rats following a Single Intravenous Injection of Daunomycin

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SUMMARY

Groups of 20 young female Sprague-Dawley rats were given a single i.v. injection of either 20, 10, or 5 mg of daunomycin per kg or of 0.9% NaCl solution. Eighteen rats given the 20-mg dose died before 5 months; 2 were killed when debilitated at 51 days and were found to have chronic glomerulonephritis. Thirty-three rats from the groups given 5 or 10 mg were available for pathological study when the experiment was terminated after 1 year. Of these animals, 16 had 27 tumors. Nineteen of the treated rats in the 5- and 10-mg groups had chronic glomerulonephritis. This was associated with secondary hyperparathyroidism manifested by parathyroid hyperplasia, metastatic calcification, and hyperostotic bone disease.

INTRODUCTION

In 1963, Grein et al. (14) reported on a new antibiotic, daunomycin, isolated from cultures of a Streptomyces. Independently, Dubost et al. (10) described rubidomycin as apparently identical to daunomycin but derived from another type of Streptomyces. Daunomycin is classified as an anthracycline and consists of 2 components: daunomycinone, a pigmented aglycone, in glycoside linkage with daunosamine, an amino sugar. Its cytotoxicity, antimitotic effects, and antitumor activity in animals led to subsequent clinical studies that have shown the drug to be a useful agent for the treatment of leukemia, particularly in children (35).

Studies of the biological and chemical activity of daunomycin have been recently reviewed (4, 9). Of special interest is the observation that daunomycin combines with DNA, presumably by intercalation between base pairs (7); it also inhibits both DNA and RNA synthesis (7). Daunomycin produces nuclear and nucleolar abnormalities similar to those seen with proflavin or ethidium bromide (29). In addition to cytotoxic effects, it has several interesting pathological effects in the whole animal. In rats, for instance, a single injection can produce severe damage to proliferative tissues (bone marrow, intestinal crypts, and lymphoid tissues). Recovery from this initial intoxication is followed by renal injury resulting in the development of a nephrotic syndrome. The nephrosis is persistent and self-perpetuating, and ultimately progresses to a chronic glomerulonephritis (32, 33). During the 1st weeks of the renal disorder, when glomerular damage is prominent, cytomegalic tubular epithelial cells appear. The presence of these atypical cells with abnormal nuclei, plus the known binding of daunomycin to DNA, led us to initiate a carcinogenesis experiment which is the principal subject of this report.

MATERIALS AND METHODS

The animals used were Sprague-Dawley virgin female rats weighing 120 to 166 g (CD line; Charles River Breeding Laboratories, Brookline, Mass.). Groups of 20 rats received 20, 10, or 5 mg of daunomycin per kg or 0.9% NaCl solution. Each animal received 1 i.v. injection via the tail vein. Animals were housed in stainless steel cages. When each weighed less than 300 g, there were 5 rats/cage, and thereafter there were 2 rats/cage. They were given Purina rat chow and water ad libitum. Animals were killed at 6 months, 2 at 10 months, and 3 at 1 year; the remaining pair was killed at Day 51 and was found to have severe chronic glomerulonephritis. In the group given 10 mg/kg, 9 rats were killed at 6 months, 2 at 10 months, and 3 at 1 year; the remaining 6 were found dead at Days 92, 175, 176, 184, 283, and 351. In the group given 5 mg/kg, 1 rat was killed at 7 months, 3 at 10 months, and 15 at 1 year; 1 was found dead at...
Day 176. All of the control rats were killed between 10 and 12 months; none were found dead. Thus, a total of 33 rats from the groups receiving doses of 5 or 10 mg/kg were available for pathological studies and, of these, 16 had 27 tumors (Table 1). In the control group, 5 of the 20 rats had single tumors (Table 1).

Tumors. Two daunomycin-treated rats had adenocarcinomas of the kidney (Table 1). One of these tumors was 2 cm in diameter and replaced a major portion of the kidney. In the other rat, 2 separate foci of adenocarcinoma were present in the same kidney; the largest measured 0.5 cm. These carcinomas had similar histological appearances and consisted of sheet-like masses of cells of the clear-cell type. The nuclei were hyperchromatic and irregular. The overall appearance of these tumors closely resembled the clear-cell carcinomas of the kidneys of humans (Figs. 1 to 3).

In addition, renal adenomas were present (Table 1), one of which was a microscopic focus of the clear-cell type (Fig. 4). The other examples contained cells of the granular type, with papillary and cystic areas. One granular adenoma was present in the same kidney that had a clear-cell carcinoma (Fig. 5). The renal cysts (Table 1) present in 2 rats were lined by a single layer of atypical cells with irregular nuclei. One cyst was composed of clear cells and the other of cells with granular cytoplasm. All the rats with tumors and cysts of the kidney had chronic glomerulonephritis. No kidney tumors were present in the control rats and none had renal disease.

Five of the daunomycin-treated rats had a total of 7 tumors involving the vagina, the uterus, or both. Four of these rats had been given daunomycin, 10 mg/kg (Table 1). There were no genital tract tumors in controls.

The other tumors listed in Table 1 showed no unusual histological features and warrant no further comment. In no instance were metastases found.

Other Findings. In a previous study, the early course of the nephrotic syndrome was followed in a group of rats given a single i.v. injection of daunomycin, 20 mg/kg. These rats were killed at Days 1, 2, and 4, as well as weekly up to 6 weeks. In addition to glomerular damage, which appeared at Day 4, tubular damage and protein casts were noted. At 1 week and thereafter, cytomegalic cells were found, and these were located primarily in tubular epithelium in various parts of the nephron but were most numerous in the papilla (Fig. 6). The enlargement of the cells was due primarily to irregular and hyperchromatic nuclei (Figs. 7 and 8); in a few such cells, inclusions were present in the nuclei (Fig. 9). While most of these cells were of tubular origin, a few were noted in the interstitial tissues (Fig. 9) and pelvic mucosa (Fig. 10).

In this study, 13 of 14 rats that received doses of daunomycin, 10 mg/kg, and 9 of 19 that received 5 mg/kg had

### Table 1

<table>
<thead>
<tr>
<th>Type of Tumor</th>
<th>No. of Tumors After Administration of</th>
<th>Daunomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg/kg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5 mg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Renal tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear-cell carcinoma</td>
<td>0</td>
<td>2 (360, 365)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tubular adenoma</td>
<td>2 (189, 366)</td>
<td>3 (294, 360, 365)</td>
</tr>
<tr>
<td>Cysts</td>
<td>2 (179, 361)</td>
<td>0</td>
</tr>
<tr>
<td>Genital tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal myoma</td>
<td>0</td>
<td>1 (361)</td>
</tr>
<tr>
<td>Cervical polyp</td>
<td>2 (303, 361)</td>
<td>0</td>
</tr>
<tr>
<td>Endometrial polyp</td>
<td>2 (182, 361)</td>
<td>0</td>
</tr>
<tr>
<td>Cervical myosarcoma</td>
<td>1 (366)</td>
<td>0</td>
</tr>
<tr>
<td>Hemangioma, uterine wall</td>
<td>1 (361)</td>
<td>0</td>
</tr>
<tr>
<td>Mammary tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>2 (193, 303)</td>
<td>4 (301, 317, 360, 365)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1 (193)</td>
<td>0</td>
</tr>
<tr>
<td>Lung tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>1 (361)</td>
<td>0</td>
</tr>
<tr>
<td>Liver tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplastic nodule</td>
<td>1 (317)</td>
<td>0</td>
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<tr>
<td>Skin tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>1 (193)</td>
<td>0</td>
</tr>
<tr>
<td>Other tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myosarcoma, inguinal region</td>
<td>0</td>
<td>1 (207)</td>
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<tr>
<td>Spindle-cell sarcoma, chest wall</td>
<td>0</td>
<td>0</td>
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</table>

<sup>a</sup> Sixteen tumors were found in 10 of 14 rats killed.
<sup>b</sup> Eleven tumors were found in 6 of 19 rats killed.
<sup>c</sup> Five tumors were found in 5 of 20 rats killed.
<sup>d</sup> Nos. in parentheses, days on which animals were killed.
chronic glomerulonephritis. These lesions were severe and involved most glomeruli (Fig. 11). Affected glomeruli were diffusely fibrotic; some had areas of fibrinoid necrosis, and others were obliterated. Many tubules were dilated and cystic, and protein casts were prominent (Fig. 11). Calcification of the interstitial tissues, tubules, and the media of arteries was also noted. The interstitial tissues were also the site of fibrosis and collections of inflammatory cells which were mainly lymphocytes; in addition, lymphoid nodules were noted in a few instances. In a number of rats, there was arteritis involving the kidney, pancreas, heart, skeletal muscle, and intestinal mesentery.

A group of 6 rats that received daunomycin, 10 mg/kg, were debilitated at about 6 months, at which time they were killed and complete autopsies were performed. All had severe chronic glomerulonephritis, and in 5 there was evidence of hyperparathyroidism secondary to chronic renal disease. This was manifested by hyperplasia of the parathyroid glands (Fig. 12), metastatic calcification involving kidneys and stomach, and bone disease. The bone changes consisted of an increase in thickness of the trabeculae and cortex, but there was no abnormal osteoclastic or osteoblastic activity or fibrous reaction (Figs. 13 and 14).

**DISCUSSION**

With the exception of a special strain of Wistar rats that develop familial renal adenomas (11), the incidence of spontaneous kidney tumors in rats is very low (30). No renal tumors were found among 150 Sprague-Dawley rats in 1 study (8), 1 nephroblastoma was noted among 125 rats in another series (36) and, more recently, 1 renal adenoma was observed among a group of 934 Sprague-Dawley rats (27). Thus, the presence of 5 adenomas and 2 clear-cell carcinomas among 33 daunomycin-treated rats, all killed at or before 1 year, represents a relatively high incidence of renal tumor formation. Conversely, mice given daunomycin by the p.o. or i.p. route showed no carcinogenic effect although, when the compound was given s.c., fibrosarcomas were found at the injection site (4).

A number of different agents have been shown to produce tumors of the kidney in rats. These include organic chemicals such as the nitrosamines (21, 38), acetylated mono- and diaminobiphenyl compounds (24), and 4'-fluoro-4-aminodiphenyl (23). The only inorganic chemicals capable of producing kidney tumors are lead salts (40). More recently, cycasin, administered once, is also capable of tumor induction (17). Likewise, a single dose of the aglycone of cycasin, methylazoxymethanol, produces kidney and other types of tumor (19), as does the acetate salt of methylazoxymethanol (39). A single p.o. administration of dimethylnitrosamine has also been shown to be carcinogenic for the rat kidney (22). The renal tumors induced in rats by these agents are not associated with either glomerulonephritis or any other noteworthy alteration in the nontumorous portion of the kidney. The association of chronic renal disease and renal tumorigenesis is an unusual one, and apparently daunomycin is unique in this respect. In humans, the association of cancer of the kidney and glomerulonephritis appears to be a random one (25). *Mastomys*, a subgenus of rodent intermediate in size between the rat and the mouse, spontaneously develops renal tumors and glomerulonephritis (31).

The cytomegalic cells were present in the kidney during the early phase of the nephrosis. When abnormal tubular cells are seen in glomerulonephritis, the change is generally considered to be secondary to glomerular ischemia (3). However, when such vascular alterations are present, most of the tubular cells in any given segment of the nephron are altered. The daunomycin-induced cytomegaly, however, affected single, isolated cells within the tubules, while adjoining cells were normal—much as viral inclusion bodies are distributed in tubular cells (3). Furthermore, cytomegalic cells were noted in the pelvic mucosa, a site that is removed from the effects of such ischemia. The development of cytomegaly has been observed with other carcinogenic agents, not only in the kidney, but in liver as well. For example, “monstrous” nuclei in the kidney of rats after the administration of dimethylnitrosamine have been described (38). Similar changes were noted in hepatocytes of Syrian hamsters treated also with dimethylnitrosamine (15). Affatoxin produces both hepatic and renal cytomegalic cells in Syrian hamsters (16). Retrorsine and other pyrrolizidine alkaloids produce similar enlarged hepatic cells in rats (1, 5). The significance of these enlarged cells is not known. Those in the liver have been interpreted as a degenerative change (6) or as possibly representing a preneoplastic proliferation (28). In a radioautographic study with retrorsine and adenine-14C, both the large and small hepatocytes were found equally able to synthesize nuclei acid. However, while the large cells were apparently capable of growth, they were incapable of division (1). More recently, from studies with lasiocarpine-induced megalocytosis and thymidine-3H, it was also concluded that these cells were incapable of division (34). Such a phenomenon would be consistent with finding that none of the daunomycin-induced tumors, either benign or malignant, contained cytomegaly cells with the degree of enlargement and atypia that were characteristic of the cells noted in the early phase of toxicity.

A moderate incidence of spontaneous genital tract tumors in the female rat has been noted in animals who have lived their life-span (12, 36). The incidence of such tumors in the daunomycin-treated rats is considerably greater, and the onset is apparently earlier. In addition, in the present series, the presence of 4 cervical tumors is unusual, since such lesions are considered a rarity in rats (12). The diversity of tumor types and their varied locations in this group of induced genital tumors suggests that they are not related to endocrine dysfunction. Rather, the acute toxic effect of daunomycin on the genital tract is a mechanism that should be considered, although it has not been, as yet.

The findings of parathyroid hyperplasia, metastatic calcification, and bone disease associated with chronic renal disease are reasonable morphological criteria for the diagnosis of secondary hyperparathyroidism. The bony lesions associated with hyperparathyroidism are usually those of osteitis...
fibrosa (2), while the less common types of involvement are either osteomalacia or, as in the present study, osteosclerosis. A similar type of excess bone production has been observed in some patients with chronic renal insufficiency (13, 18). Whereas in osteitis fibrosa the demineralization of bone is attributed to the effects of parathormone, and the lesions of osteomalacia are attributed to dietary levels of vitamin D and calcium, the mechanism of the development of osteosclerosis remains obscure (18). The possibility that the hormone calcitonin may play a role in such bone disease is suggested by its action in enhancing the incorporation of calcium into bone (26, 37) although the suppression of osteolysis is thought to be its main action.

ADDENDUM

A recently reported carcinogenesis experiment in which single i.v. doses of daunomycin and adriamycin were used revealed 11 mammary adenocarcinomas and 2 fibroadenomas in 25 daunomycin-treated rats and 1 adenocarcinoma and 6 fibroadenomas in 25 adriamycin-treated rats at the end of 1 year. Also found in the daunomycin-treated rats were 1 rhabdomyosarcoma and 1 uterine polyp; and in the adriamycin group, 1 meningioma and 2 uterine polyps were found. No renal tumors were reported, and none of the 25 control rats showed tumors. (C. Bertazzoli, T. Chieli, and E. Solcia. Different Incidence of Breast Carcinomas or Fibroadenomas in Daunomycin or Adriamycin Treated Rats. Experientia, 27: 1209–1210, 1971.)

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Fig. 1. Representative section of large (2-cm) clear-cell adenocarcinoma of the kidney of a rat killed at 1 year. H & E, X 110.

Fig. 2. One of 2 clear-cell adenocarcinomas present in a rat killed at 360 days. This rat also had chronic renal disease (see Fig. 11). H & E, X 110.

Fig. 3. High-power view of the tumor from rat shown in Fig. 2. Note the irregular hyperchromatic nuclei and mitotic figure (arrow). H & E, X 400.

Fig. 4. Example of a small adenoma of the clear-cell type present in the kidney of a rat killed at 1 year. H & E, X 140.

Fig. 5. Example of a granular adenoma of the kidney in a rat killed at 360 days (the same rat that had a clear-cell adenocarcinoma; Figs. 2 and 3). H & E, X 400.

Fig. 6. Section of the renal papilla of a rat killed 4 weeks after it received a single i.v. injection of daunomycin, 20 mg/kg. Many of the tubular cells and nuclei are enlarged. H & E, X 400.

Fig. 7. Example of marked nuclear enlargement of an epithelial cell in a distal tubule of the cortex. The other nuclei of this tubule are normal. This rat was killed 4 weeks after receiving an i.v. injection of daunomycin, 20 mg/kg. H & E, X 1000.

Fig. 8. Another example of nuclear enlargement of a tubular epithelial cell in a rat killed 6 weeks after receiving an i.v. injection of daunomycin, 20 mg/kg. Note the protein cast in tubule at upper right. H & E, X 400.

Fig. 9. An enlarged nucleus in an interstitial cell. Note the presence also of an intranuclear inclusion (same rat as in Fig. 8). H & E, X 400.

Fig. 10. Example of an enlarged nucleus in the pelvic mucosa of a rat killed 4 weeks after receiving an i.v. injection of daunomycin, 20 mg/kg. H & E, X 400.

Fig. 11. Example of chronic renal disease in a rat killed 360 days after receiving a single injection of daunomycin, 5 mg/kg. The glomeruli and interstitium show fibrosis; many tubules are dilated and contain protein casts (same rat as in Figs. 2 and 3). H & E, X 125.

Fig. 12. Example of an enlarged parathyroid in a rat with chronic renal disease, killed 189 days after receiving a single i.v. injection of daunomycin, 10 mg/kg. H & E, X 125.

Fig. 13. Section of the sternum in a rat with chronic renal disease killed 176 days after receiving a single i.v. injection of daunomycin, 10 mg/kg. Note the marked osteosclerosis and attenuated marrow spaces. Compare with normal sternum from a control animal (Fig. 14). H & E, X 125.

Fig. 14. Normal sternum of a control rat, for comparison with Fig. 13. H & E, X 125.
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